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3,528,888

## REAGENT AND METHOD FOR ASSAYING ALPHA-HYDROXYBUTYRATE DEHYDROGENASE

Alfred Deutsch, Los Angeles, Calif., assignor to Calbiochem, Los Angeles, Calif., a corporation of California  
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3 Claims

### ABSTRACT OF THE DISCLOSURE

Substantially anhydrous, solid assay materials for the determination, inter alia, of Reagent for Assaying Alpha-Hydroxybutyrate Dehydrogenase, are rendered storage stable by the presence of certain polyhydric compounds preferably mannitol, sorbitol, lactose or polyvinyl alcohol.

This application is a divisional of my copending application Ser. No. 561,757, filed June 30, 1966 now U.S. Pat. 3,413,198, which in turn is a continuation-in-part of my copending application Ser. No. 320,004, filed Oct. 30, 1963 and now abandoned.

The present invention relates to processes and compositions for preparing reagent mixtures for detecting and measuring the presence of certain components in a biological sample. It also relates to the novel reagent mixtures.

In the clinical diagnosis of certain pathological conditions, it is frequently valuable to know the amount of activity or the quantity of certain substances present in a specimen of a biological or other fluid or tissue. One of the more effective means that has been proposed for making assays of such specimens is to provide a liquid reagent which contains one or more biological components. When a given reagent is mixed with the specimen, the components are effective to cause an enzymatic reaction that involves the unknown substance. By observing this reaction, it is possible to determine the quantity or amount of activity of the unknown originally present.

Since such reagents contain one or more biological components such as enzymes, coenzymes and/or substrates, etc., the reagent has inherently been of a very unstable nature and has very little if any shelf life. To insure the reagent being at optimum strength it must be prepared at or immediately prior to the time the assay is made. In addition, heretofore the various components such as the enzymes, coenzymes, substrates, etc., included in the reagent have been very unstable. To insure these components being at their optimum it has been necessary for the components to be stabilized in a concentrated form.

When it has been desired to make a biological assay of the present type, a "kit" containing the several different components which may be dry, or in solutions, has been obtained. If the components are in a dry form, aqueous solutions are formed, and maintained separately until just prior to use.

The various components for the reagent are present in separate containers and maintained separated from each other. Some of these solutions and particularly those containing the enzymes are necessarily in a concentrated form in order to preserve their activity.

When employing a "kit" of this type, to assay a specimen, it is necessary to first reconstitute the components to the required strength by adding a specified amount of another liquid such as water to various solutions. After all of the various components have been reconstituted, the appropriate quantities of each are combined to form

the reagent. A predetermined quantity of the reagent is then mixed with the specimen to produce the desired assay reactions. The accuracy of the final assay is also dependent upon the accuracy with which components are reconstituted, the accuracy with which the reconstituted components are combined to form the resultant reagent and the accuracy with which the reagent is measured when it is mixed with the specimen. It may thus be seen that the accuracy of the assay is dependent upon the skill of the operator and the accuracy with which he prepares and uses the reagent.

It can be readily appreciated that the foregoing process is very time-consuming particularly when considering the time for using and cleaning the substantial amounts of equipment such as various pieces of glassware, measuring instruments, etc. If any of the equipment has any foreign matter thereon, the reagent may easily be contaminated whereby the results of the assay will be misleading.

It should also be noted that after the reagent is fully prepared, at least one of the components therein and particularly the enzymes are quite unstable and rapidly lose their activity. As a consequence, if the reagent is not used within a matter of a few hours following its preparation, it must be discarded and, therefore, wasted. The percentage of the reagent wasted in this manner becomes very large where only a few assays are made at infrequent intervals.

It may thus be seen that although the foregoing "kits" have been capable of producing the desired reactions and permitting the desired assays to be made they have not been entirely satisfactory for numerous reasons. For example, they have not only been very time-consuming and wasteful, but have also required a person of sufficient skill to insure the accurate preparation of the reagents and their being used in the proper manner. Also, because of the possibility of substantial human errors such reagents have induced a certain degree of unpredictable error in the results of the assay.

It is an object of the present invention to provide means which will be effective to overcome the foregoing difficulties. More particularly, it is proposed to provide new and novel assay materials useful in making biological assays and the method for preparing the materials. All of the assay materials are in a dry, solid state that may be easily handled and used. The assay materials include components such as enzymes, coenzymes and/or substrates which have heretofore been very unstable. Moreover, the combining of such components tends to reduce their stability. However, stabilizers are included that are effective to maintain or preserve the activity of each of the components and of the entire assay material. Each of the components including those containing the enzymes may be stabilized individually and used as such for any desired purpose. Also, the compounds may be combined together to form a new and novel assay material. The resultant assay material contains all of the components except water, for making a liquid reagent that can be used to make a biological assay of the above described type.

Although the assay material contains various components such as enzymes, coenzymes and/or substrates, etc., the material is in a dry, solid form and will be very stable and have a long shelf life. This will permit the assay material to be packaged into containers which are easy to handle and use. Each of the containers may include a quantity of the assay material that is just the right amount for making a particular number of assays, for example a single assay. The assay material may desirably include an agent which, among other things, is effective to increase the volume of the assay material to a standard size whereby the quantity of the assay material required to produce a single assay will always be a standard